In re application of GAGE, FRED et al

Application No.: 09/421,971

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In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 6, line 29 to page 7, line 10 and replace it with the following paragraph:

Atty. Dkt. No. SALK2350

(088802-5351)

Figure 1 is a schematic diagram of [[an]] a nucleic acid construct encoding invention fusion proteins that contain EcR (darkly shaded) with a dimer partner, U (Usp) or R (RXR) (darkly shaded). "D" = DNA binding domain; "L" = ligand binding domain; curvilinear line = fusion bridge. "Individual" (SEQ ID NOs: 76 & 77 which encode SEQ ID NO: 79) represents a nucleotide sequence that encodes the wild type C terminus of EcR (receptor) and the monomeric N-terminus of RXR (binding partner) before introduction of a nucleotide sequence encoding a fusion bridge. "Fused" (SEQ ID NO: 78) represents the same segments with nucleotides inserted that encode a 5 amino acid fusion bridge (SEQ ID NO: 80) containing the Sfil insertion site. "Tether" (SEQ ID NOs: 30 & 31) indicates a nucleotide sequence that encodes a 12 amino acid linker (SEQ ID NO: 15) to be inserted into the Sfil site of the fusion bridge to produce fusion proteins with greater spacing between the two protein units (i.e., dimer partners) in the invention fusion protein.

Please delete the paragraph on page 7, lines 15-22 and replace it with the following paragraph:

Figure 2A is a graph quantifying gel mobility shift as a result of response element binding to invention endodimer FDs in the presence of murA. Controls were treated either with vehicle (open bars, i.e., -MurA) or with 1 µM murA as ligand (black bars, i.e., + MurA). Bars are labeled along the bottom with FDs named as described in the text. E represents an EcR only control; NON represents a non-transfected control. E+U and E+R

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are control lanes of monomeric in vitro translated proteins used for sizing of endodimer band shifts. Numbers at the top of each bar represent relative-fold increase in response element binding resulting from ligand treatment.

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Please delete the paragraph on page 8, lines 3-8 and replace it with the following paragraph:

Figure 3 is a graph showing relative luciferase expression induced by FD constructs with or without ligand as determined in transient transfection assays for FDs and for monomeric EcR with either Usp or RXR when treated with vehicle (open bars, i.e., -MurA) or 1 µM murA MurA (black bars, i.e., + MurA). Decimal numbers on the abscissa represent molar amount of FD relative to VE plasmid (1.0 is equimolar FD:VE). EOU and UOE without VE cotransfection are at the extreme right of the bar graph. See also Table 1.

Please delete the paragraph on page 8, lines 9-21 and replace it with the following paragraph:

Figures 4A-B are two graphs illustrating the results of transient transfection assays conducted using either VP16-fused monomeric receptors or invention fusion protein FDs with increasing 'linker lengths. Figure 4A is a graph showing a comparison of luciferase activity in relative light units (RLU) in transient transfection assays conducted with or without ligand, using either monomeric receptors having amino terminal fused VP16 activation domains or invention FDs containing EcR, RXR, and a linker with a variable number of linker segments. Cells were treated with vehicle (open bars, i.e., -MurA), or 1 µM muristerone A as ligand (black bars, i.e., + MurA). Numbers at the top of the bars indicate the fold-increase relative to FD or monomeric receptor without addition of monomeric VRXR (VR) or monomeric VUsp (VU). E = EcR only; E4-luc = reporter plasmid only; and Figure 4B is a graph showing a comparison of luciferase activity as in Figure 4A herein, except that the FDs contain Usp in place of RXR.

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Please delete the paragraph on page 8, line 22 to page 9, line 4 and replace it with the following paragraph:

Figure 5 is a series of three graphs showing repression of ligand-stimulated luciferase expression by monomeric receptors caused by competition with the invention ENU and UNE FDs when transiently co-transfected into 293 cells and treated either with vehicle (open bars, i.e., -MurA), or 1 μM muristerone A as ligand (black bars, i.e., + MurA). Decimal numbers on the abscissa represent molar amount of FD relative to VE plasmid (1.0 is equimolar FD:VE). Figure 5A shows a comparison of the inhibitory effects of E0U and U0E on ligand-stimulated expression of luciferase by monomeric VE in combination with endogenous RXR. E0U and U0E without YE cotransfection are at the extreme right of the bar graph; Figure 5B shows the effects of monomeric EcR (without VP16 fusion) on ligand-stimulated expression of luciferase in the assay of Figure 5A by competition with EOU or U0E; and Figure 5C shows the effects of monomeric EcR on luciferase expression in the presence of ligand in the assay of Figure 5B, as compared with VE combined with monomeric exogenous Usp.

Please delete the paragraph on page 9, lines 5-11 and replace it with the following paragraph:

Figure 6 is a graph showing a comparison of results (in RLU) obtained in assays in which E5U and E5R compete with monomeric VRXR (VR) or monomeric VUsp (VU) in the presence of vehicle only (open bars, i.e., -MurA) or murA as ligand (black bars, i.e., +MurA). FDs and receptor combinations are labeled along the abscissa. Numbers above the bars represent the fold-increase relative to FD or receptor without addition of VR or VU. E4LUC at the extreme right is reporter plasmid alone as control.